A single amino acid substitution in the MurF UDP-MurNAc-pentapeptide synthetase renders Streptococcus pneumoniae dependent on CO2 and temperature

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Figure S1

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BHN100          MKLTIHEIAQVVGAKNDISIFEDTQLEKAEFDSRLIGTGDLFVPLKGARDGHDFIETAFE 60
Spain9V-3       MKLTIHEIAQVVGAKNDISIFEDTQLEKAEFDSRLIGTGDLFVPLKGARDGHDFIETAFE 60
                *******:*****.********************* ******************: ***

BHN100          NGAAVTLSEKEVSNHPYILVDDVLTAFQSLASYYLEKTTVDVFAVTGSNGKTTTKDMLAH 120
Spain9V-3       NGAAVTLSEKEVSNHPYILVDDVLTAFQSLASYYLEKTTVDVFAVTGSNGKTTTKDMLAH 120
                **********:*:***************:*::******:********************:

BHN100          LLSTRYKTYKTQGNYNNEIGLPYTVLHMPEGTEKLVLEMGQDHLGDIHLLSELARPKTVI 180
Spain9V-3       LLSTRYKTYKTQGNYNNEIGLPYTVLHMPEGTEKLVLEMGQDHLGDIHLLSELARPKTAI 180
                ***  ************************************************:***.*

BHN100          VTLVGEAHLAFFKDRSEIAKGKMQIADGMASGSLFLAPADPIVEDYLPTDKKVVRFGQGA 240
Spain9V-3       VTLVGEAHLAFFKDRSEIAKGKMQIADGMASGSLLLAPADPIVEDYLPIDKKVVRFGQGA 240
                **************************** ****:* ** .******  ***********

BHN100          ELEITDLVERKDSLTFKANFLEQVLDLPVTGKYNATNAMIASYVALQEGVSEEQIRLAFQ 300
Spain9V-3       ELEITDLVERKDSLTFKANFLEQALDLPVTGKYNATNAMIASYVALQEGVSEEQIRLAFQ 300
                ** **************.*****.* ****************************: ***

BHN100          HLELTRNRTEWKKAVNGADILSDVYNANPTAMKLILETFSAIPANEGGKKIAVLADMKEL 360
Spain9V-3       HLELTRNRTEWKKAANGADILSDVYNANPTAMKLILETFSAIPANEGGKKIAVLADMKEL 360
                 *************.*******************************************

BHN100          GDQSVQLHNQMILSLSPDVLDIVIFYGEDIAQLAQLASQMFPIGHVYYFKKTEDQDQFED 420
Spain9V-3       GDQSVQLHNQMILSLSPDVLDIVIFYGEDIAQLAQLASQMFPIGHVYYFKKTEDQDQFED 420
                *:**:**************** *****::**:****.**********************

BHN100          LVKQVKESLGAYDQILLKGSNSMNLAKLVESLENEAK 457
Spain9V-3       LVKQVKESLGAYDQILLKGSNSMNLAKLVESLENECK 457
                *********.. ************** *** **** *
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Fig. S1 Alignment of the BHN100 and Spain^{9V}-3 MurF amino acid sequences. MurF of 34 *S. pneumoniae* strains in the NCBI genome database were aligned with clustalW. The outcome of this alignment is give below the BHN100 and Spain^{9V}-3 MurF sequences: '*****', absolutely conserved; ':', highly conserved; and, '.', conserved. Highlighted amino acids are different between BHN100 and Spain^{9V}-3: black, unique to BHN100 and SP9-BS68; grey, not unique to BHN100 and the SP9-BS68.

Figure S2

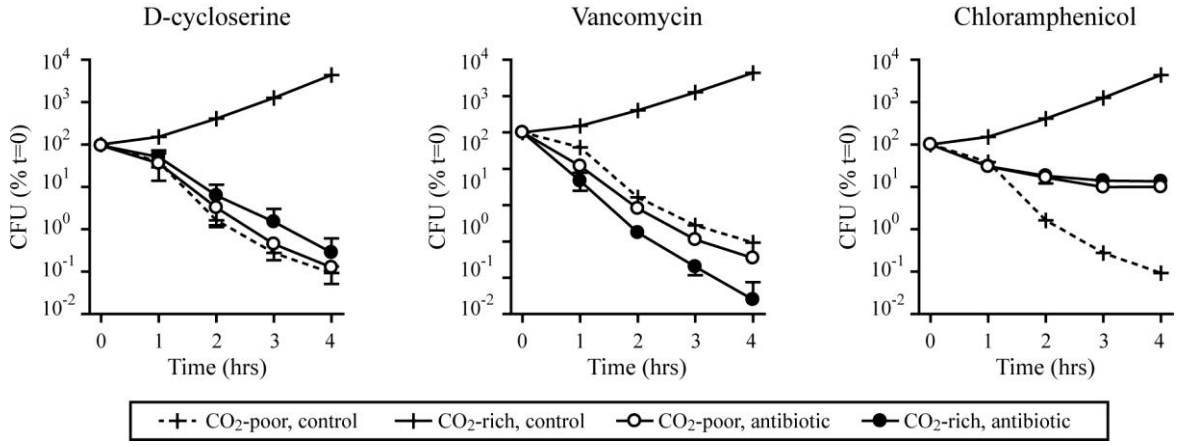

Fig. S2. Antibiotic treatment of the BHN100 strain. *S. pneumoniae* BHN100 MurFV[179] cultures were incubated in BHI broth exposed to CO_2-poor ambient air or 5% CO_2-enriched conditions in the presence or absence of the 50 µg ml^{-1} D-cycloserine, 2 µg ml^{-1} vancomycin, or 34 µg ml^{-1} chloramphenicol. Bacterial growth and survival was determined by bacterial viable counts. Count data is presented as mean values (n≥3) with standard deviation.

Figure S3


Streptococcus_pneumoniae_BHN100 -----MKLTIHEIAQVVGAKNDISIFED---TQLEKAEFDSRLIGTG--- 39
Streptococcus_pneumoniae_SP195 -----MKLTIHEIAQVVGAKNDISIFED---TQLEKAEFDSRLIGTG--- 39
Pseudomonas_putida_F1 -----MLKPLSLSQLTTALKGQHIGGD----ATFTGVSIDSRSVSAG--- 38
Pseudomonas_syringae_pv._tomat -----MIKPMTFSELTVPLDARLVGGD----CSFDGVSTDSRGIAPG--- 38
Bartonella_henselae_str._Houst -----MKALWDKQALITAIDGYAIGEIP---ETFSGISIDSRTLIEG--- 39
Borrelia_burgdorferi_80a ------MRIKIKDILISSKDVKFVGNIKNIERVVSFYSLDSREIKDDNI- 43
Helicobacter_pylori_B8 MQSLSWLNLAFRWLFITGLGYYIMTLLQWYHYSVFRILTKHHKMRWHGIY 50
Streptococcus_pneumoniae_BHN100 ------DLFVPLKGAR-DGHDFIETAFENGAAVTLSEK---------- 70
Streptococcus_pneumoniae_SP195 ------DLFVPLKGAR-DGHDFIETAFENGAAVTLSEK---------- 70
Pseudomonas_putida_F1 ------QLFVALAGPRFDGHDYLADVKAKGAVAALVER---------- 70
Pseudomonas_syringae_pv._tomat ------QLFVALTGPRFDGHEYLDQVAAKGAVGALVEH---------- 70
Bartonella_henselae_str._Houst ------DIFFCIKGHHLDGHDFAVQAYERGAAVLIVAENRL--------- 74
Borrelia_burgdorferi_80a ----NDSLYFAYKGNKVDGFSFVKYLIDLGVKCFACSREHESECIK---- 85
Helicobacter_pylori_B8 FLLPLGVFILSYAFKMPFVFDFFCGVIQMPMLIVWAKRNDKPLVFTPRVK 100
Streptococcus_pneumoniae_BHN100 ----------EVSNHPYILVDDV 83
Streptococcus_pneumoniae_SP195 ----------EVSNHPYILVDDV 83
Pseudomonas_putida_F1 ----------EVAGVDLPQLLVKGC 85
Pseudomonas_syringae_pv._tomat ----------EVEGAALPQLVVLDT 85
Bartonella_henselae_str._Houst ----------ADMEKISAPLIVVSDV 90
Borrelia_burgdorferi_80a ----------YLNDNEGLVFLLTSNV 101
Helicobacter_pylori_B8 RFFIFLLLFLILHEILNTELVPLSGISLALGYLCLFIFVLSASLIFEKAL 150
Streptococcus_pneumoniae_BHN100 LTAFQSLASYYL-EKTTVDVFAVTGSNGKTTTKDMLAHLLSTRYKTYKTQ 132
Streptococcus_pneumoniae_SP195 LTAFQSLASYYL-EKTTVDVFAVTGSNGKTTTKDMLAHLLSTRYKTYKTQ 132
Pseudomonas_putida_F1 RVALGQLGALNR-AAFDKPVVAITGSSGKTTVKEMLASILRTRGLVHATR 134
Pseudomonas_syringae_pv._tomat RKALAQLGAMNRNGFVDRPVAAVTGSSGKTTVKEMLASILRTRGPVLATR 135
Bartonella_henselae_str._Houst LQALEKLAQAAR-KRSKAKIIAVTGSVGKTTTKEVLKQVLATVGKVHANS 139
Borrelia_burgdorferi_80a IKLLQALASFLI-EKTSFKRIAITGSNGKTTTKEMLYSILSKKYKTYKTW 150
Helicobacter_pylori_B8 SKQYLQTAKDKIASLKNLKVIAITGSFGKTSTKNFLLQILQATFNAHASP 200
Streptococcus_pneumoniae_BHN100 GNYNNEIGLPYTVLH-MPEGTEKLVLEMGQDHLGDIHLLSELARPKTVIV 181
Streptococcus_pneumoniae_SP195 GNYNNEIGLPYTVLH-MPEGTEKLVLEMGQDHLGDIHLLSELARPKTAIV 181
Pseudomonas_putida_F1 GNLNNDLGAPLTLLE-IAPEHSAAVIELGASRIGEIRYTVGLTQPQVVII 183
Pseudomonas_syringae_pv._tomat GNLNNELGVPLTLLE-LAPEYTSAVIELGASRVGEIAYTVSLTKPHVVII 184
Bartonella_henselae_str._Houst ASLNNHWGVPLTLAR-MPVESNYGVFEIGMNHKDEIRPLVKLVHPHVVLI 188
Borrelia_burgdorferi_80a GNLNSDIGLPLSILR-VEGNEEYAVFEVGVSYVGEMDLLSQILKPEIVII 199
Helicobacter_pylori_B8 KSVNTLLGLANDINQNLDDRSEIYIAEAGARNKGDIKEITHLIEPHLVVV 250
Streptococcus_pneumoniae_BHN100 TLVGEAHLAFFKDRSEIAKGKMQIADGMASGSLFLAPADPIVEDYLPTDK 231
Streptococcus_pneumoniae_SP195 TLVGEAHLAFFKDRSEIAKGKMQIADGMASGSLLLAPADPIVEDYLPIDK 231
Pseudomonas_putida_F1 NNAGTAHVGEFGGPDKIVEAKGEILEGLGEGGIAILNLDDKAFDIWKARA 233
Pseudomonas_syringae_pv._tomat TNAGTAHVGEFGGPERIVEAKGEILEGVEASGTAVLNLEDK---IWRKRA 231
Bartonella_henselae_str._Houst THISAAHMGFFKNLEEIADAKAEIFEGLDEEGIAVLNADDDFFPYLVQKA 238
Borrelia_burgdorferi_80a TNISYAHMQAFKELQAIAFEKSKIIG--KNIEIFVVNEMNDYCVYLEKRA 247
Helicobacter_pylori_B8 AEVGEQHLEYFKTLENICETKAELLDS-KRLEKAFCYSVEKIKPYAPKDS 299
Streptococcus_pneumoniae_BHN100 KVVR----------FGQGAELEITDLVERKDSLTFKANFLEQVLDLPV 269
Streptococcus_pneumoniae_SP195 KVVR----------FGQGAELEITDLVERKDSLTFKANFLEQALDLPV 269
Pseudomonas_putida_F1 GAH---KVVSFARSNPKADFHASDIGRDARGCPSFTLHGAGETVAVQLNV 280
Pseudomonas_syringae_pv._tomat GPR---NILTFALADAAADFHAGELSRDARGCPSFTMASPLGSAQVQLNL 278
Bartonella_henselae_str._Houst KQCSVKRILSFG-ETKNSDYQAKDIR-LLTDYSSMIVSISGQDRAMKIGA 286
Borrelia_burgdorferi_80a KIAN------PNVKIVYFDFENLSIKSFSFLEGKFSFDFVYKGFEYSILL 291
Helicobacter_pylori_B8 PLID----------VSSLVKNIQSTLKGT-SFEMLLDSVWESFETKV 335
Streptococcus_pneumoniae_BHN100 TGKYNATNAMIASYVALQEGVSEEQIRLAFQHLELTRNR----TEWKKAV 315
Streptococcus_pneumoniae_SP195 TGKYNATNAMIASYVALQEGVSEEQIRLAFQHLELTRNR----TEWKKAA 315
Pseudomonas_putida_F1 LGEHNVSNALAAAAAAHAVGLSLSGIAAGLAAVQPVKGR----TVAQIAP 326
Pseudomonas_syringae_pv._tomat LGTHNVTNALAAAAAACALGVSLEGIVAGLNNVQPVKGR----AVAQIAS 324
Bartonella_henselae_str._Houst PGRHIIQNSLAVIAACDAIGVDLEPVLLSLSYFSPQKGRGVRYRLSLPSG 336
Borrelia_burgdorferi_80a LGRHNIFNAIGCINLALFLGMREKEIKEGLIETAFQKGR-----AEILTK 336
Helicobacter_pylori_B8 LGEFSAYNIASAILIAKHLGLETERIKRLVLELNPIAHR-----LQLLEV 380
Streptococcus_pneumoniae_BHN100 NGADILSDVYNANPTAMKLILETFSAIPANEGGKKIAVLADMKELGDQSV 365

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Streptococcus_pneumoniae_SP195      NGADILSDVYNANPTAMKLILETFSAIPANEGGKKIAVLADMKELGDQSV 365
Pseudomonas_putida_F1               NGVRVIDDSYNANPTSMCAAIDILAG----FSGRTVLVLGDIGELGQWAE 372
Pseudomonas_syringae_pv._tomat      NGVRVIDDTYNANPGSINAAVDILTG----FTGRTVLVLGDIGELGEWAE 370
Bartonella_henselae_str._Houst      GEFYLIDESYNANPASMRAALDLLAIGPVGVEGRRIAILGDMLELGAYSE 386
Borrelia_burgdorferi_80a            NGYLILNDSYNGNMGSFMALKNMILD--LDIQSKKFIVLGSFKELGELAY 384
Helicobacter_pylori_B8              NQKIIIDDSFNGNLKGMLEGIRLASL----HKGRKVIVTPGLVESN---- 422
                                    .   ::.: :*.*  .:              .: . :  .: * .

Streptococcus_pneumoniae_BHN100     QLHNQMILSLSPDVLDIVIFYGEDIAQLAQLASQMFPIGHVYYFKKTEDQ 415
Streptococcus_pneumoniae_SP195      QLHNQMILSLSPDVLDIVIFYGEDIAQLAQLASQMFPIGHVYYFKKTEDQ 415
Pseudomonas_putida_F1               EGHRQVG-DYARGKVDALYAVGSNMTHAVKAFGANG---------RHFAT 412
Pseudomonas_syringae_pv._tomat      QGHHDVG-AYAAGKVSALYAVGPLMTHAVSAFGEHA---------YHFAS 410
Bartonella_henselae_str._Houst      KLHRDLVKPVYLSGANPVFLFGEAMKFLATDLAAYV---------KVHYA 427
Borrelia_burgdorferi_80a            KTHKDLILEAISMNFDKIFLIGEEFLDVRDSENLVEK--------CLYYF 426
Helicobacter_pylori_B8              TESNETLAQKIDGVFDVAIITGELNSKTIASQLKTP---------QKILL 463
                                       .:          .     *

Streptococcus_pneumoniae_BHN100     DQFEDLVKQVKESLGAYDQILLKGSNSMNLAKLVESLENEAK---- 457
Streptococcus_pneumoniae_SP195      DQFEDLVKQVKESLGAYDQILLKGSNSMNLAKLVESLENECK---- 457
Pseudomonas_putida_F1               Q--AELIDAVSAENASDTTILIKGSRSAAMENVVAALCGASGEKH- 455
Pseudomonas_syringae_pv._tomat      Q--AELIAALGAEQDPNTTLLIKGSRSAAMENVVAALCGSSLEKH- 453
Bartonella_henselae_str._Houst      ENIEKILPLILAEISNGDLLMIKSSHSLYSSDIVSALLDRYKVVSL 473
Borrelia_burgdorferi_80a            SEFDKFIDFFLKSLEPSVFIVIKGSRFNRLERILNYI--------- 463
Helicobacter_pylori_B8              KDKAQLENILQATTIQGDLILFANDAPNYI---------------- 493
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Fig. S3 Alignment of the MurF sequence from various baceterial species. MurF of *S. pneumoniae* Spain^{9V}-3, BHN100. *Helicobacter pylori*, *Pseudomonas sp*., *Borrelia sp.*, and *Bartonella sp* strains in the NCBI genome database were aligned with clustalW. The outcome of this alignment is give below the BHN100 and Spain^{9V}-3 MurF sequences: '*', absolutely conserved; ':', highly conserved; and, '.', conserved. The amino acid position equivalent to position 179 in *S. pneumoniae* MurF is highlighted.

Table S1. Primers used in this study

Primer name	Nucleotide sequence (5'-3')
PBddl1	AGAGATGTGGCAGCCATGG
PBddl2	GTGTAAGGAAGGCCAATCTC
PBMrTn1	CTAgCgACgCCATCTATgTg
PBMrTn8	CGGGAATCATTTGAAGGTTGG
PBmurF1	CCTCTTGGGTAACTACGATG
PBmurF2	CTCTGTAGTTGGAAACGATTG
PBmurF3	CTAGAGTTCTCGGACTTCAG
PBmurF4	TAGTCCAGTATCTGTAAGAGC
PBmurF5	GAGATTGGCCTTCCTTACAC
PBmurF8	ACAGCCATCGTGACCTTGGTTGGAG
PBmurF9	CCAAGGTCACGATGGCTGTTTTTGGACGAGCC
PBmurF10	CTTCAGGTTCCTTGCTTTTAGCGCCGGCTGACC
PBmurF11	CGCTAAAAGCAAGGAACCTGAAGCCATTC
PBmurF12	GAAGAAAGCAGCCAATGGAGCAGATATCCTG
PBmurF13	GCTCCATTGGCTGCTTTCTTCCACTCGG
PBmurF14	CCACTAGTTCTAGAGCGGCTGAAGTCCGAGAACTCTA
PBmurF15	GCGTCAATTCGAGGGGTATCCAAGTTGGTAGAAAGTTTAGA
PBmurF16	GTAGCCATGGAATTAACAATCCATGAAATTGCC
PBmurF17	CTTAGGATCCTCACTTGGCTTCATTTTCTAAAC
PBmutT1	AGAAGGAGTTTCAGAGGAGC
PBmutT2	GGTAAGCATCTGGCACTGG
PBpbp2b1	CTTCTGAGGACTCGTTTGG
PBpbp2b2	GGCTTGAGCAAACGTGAAAG
pbp2b-F	GAT CCT CTA AAT GAT TCT CAG GTG G
pbp2b-R	CAA TTA GCT TAG CAA TAG GTG TTG G
PBrecR1	TCACCAATTCCTTTGGGCAG
PBrecR2	ACTTCCAAGAATCCTTGAAC

Table S2. MurF amino acid position 179, 215, and 315 in non-capnophilic BHN100 and AHOY490 mutants

Strain	Donor DNA	MurF179	MurF215	MurF315
Spain^{9V}-3	reference DNA	Ala	Leu	Ala
BHN100	reference DNA	**Val**	Phe	Val
BHN100	Spain^{9V}-3 *murF* (1)	Ala	Phe	Val
BHN100	Spain^{9V}-3 *murF* (2)	Ala	Phe	Val
BHN100	BHN100 *murF* (1)	**Val**	Phe	Val
BHN100	BHN100 *murF* (2)	**Val**	Phe	Val
BHN100	BHN100 *murF*^{V179A} (1)	Ala	Phe	Val
BHN100	BHN100 *murF*^{V179A} (2)	Ala	Phe	Val
BHN100	BHN100 *murF*^{F215L} (1)	**Val**	Phe	Val
BHN100	BHN100 *murF*^{F215L} (2)	**Val**	Phe	Val
BHN100	BHN100 *murF*^{V315A} (1)	Ala	Phe	Val
BHN100	BHN100 *murF*^{V315A} (2)	**Val**	Phe	Val
AHOY490	reference DNA	**Val**	Leu	Ala
AHOY490	Spain^{9V}-3 gDNA (1)	Ala	Leu	Ala
AHOY490	Spain^{9V}-3 gDNA (2)	Ala	Leu	Ala
AHOY490	Spain^{9V}-3 gDNA (3)	Ala	Leu	Ala
AHOY490	Spain^{9V}-3 gDNA (4)	Ala	Leu	Ala
AHOY490	Spain^{9V}-3 gDNA (5)	Ala	Leu	Ala
AHOY490	Spain^{9V}-3 gDNA (6)	Ala	Leu	Ala
AHOY490	Spain^{9V}-3 gDNA (7)	Ala	Leu	Ala
AHOY490	Spain^{9V}-3 gDNA (8)	Ala	Leu	Ala
AHOY490	BHN100 gDNA (1)	**Val**	Leu	Ala
AHOY490	BHN100 gDNA (2)	Gly	Leu	Ala
AHOY490	BHN100 gDNA (3)	Gly	Leu	Ala

Movies S1. *S. pneumoniae* BHN100 MurF^{V179} cells were precultured at 30°C and spotted on 1.5% low-melting agarose containing C+Y medium as described in the Experimental Procedures. Cells were imaged at timely intervals by automated phase-contrast microscopy. After 1.5 hrs the temperature in the environmental chamber was shifted from 30°C to 37°C (visible by cells drifting out of focus) and imaging was continued for another 2 hrs. Note that the shift after 1.5 hrs corresponds to T=0 in Fig. 6A and 6B.

Movies S2. *S. pneumoniae* BHN100 MurF^{V179A} cells were precultured at 30°C and spotted on 1.5% low-melting agarose containing C+Y medium as described in the Experimental Procedures. Cells were imaged at timely intervals by automated phase-contrast microscopy. After 1.5 hrs the temperature in the environmental chamber was shifted from 30°C to 37°C (visible by cells drifting out of focus) and imaging was continued for another 2 hrs. Note that the shift after 1.5 hrs corresponds to T=0 in Fig. 6A and 6B.